OBSERVATIONS ON THE NUTRITION OF THE RHYNCHOCOELAN LINEUS RUBER (O. F. MÜLLER)

J. B. JENNINGS

Department of Zoology, University of Leeds, England

Feeding and digestion in the Rhynchocoela have received relatively little attention apart from brief accounts by Wilson (1900), Reisinger (1926), Coe (1943) and Hyman (1951). These indicate that the group is carnivorous, preying upon a variety of invertebrates which are captured by means of the extensible proboscis and swallowed whole, and that digestion may be either extracellular or partially intracellular. No further details of rhynchocoel nutrition are available and to remedy this deficiency, that of the common British species *Lineus ruber* (O. F. Müller) has been investigated.

Materials and Methods

Specimens of *Lineus ruber* were collected from beneath rocks embedded in sand at mid-tide level at Cremyll, Plymouth. After starvation for two days to induce a readiness to feed and to clear the gut of remnants of previous meals, individuals were presented with representatives of the fauna of their habitat, and the methods of capture and ingestion of the selected prey observed.

The course of digestion was followed by histological examination of individuals fixed at progressive intervals after feeding upon either the natural food or easily identifiable test foods such as frog erythrocytes and raw, optically active starch grains. Fixation was in Susa at 30° C. and sections cut at 8 μ were stained with the haematoxylin and eosin, Feulgen, periodic acid-Schiff (P.A.S.), Alcian blue (for mucin), benzidine-peroxide (for haemoglobin), and Lugol's iodine techniques. Changes in the pH of the gut contents during digestion were followed by feeding particles of fish muscle stained with 0.5% sea water solutions of various indicators and observing subsequent color changes by periodically flattening the fed individuals and examining by both reflected and transmitted light.

Food reserves were studied after fixation in Flemming (for fats) and 90% alcohol containing 1% picric acid (for carbohydrates and proteins). Sections of individuals fixed in the latter reagent were stained by the Best's carmine, P.A.S. and modified Millon's methods.

Observations

The food and feeding mechanism

Lineus ruber feeds mainly on small annelids and crustaceans but particles of any dead organic material will be taken, providing it is not too decomposed. The oligochaete *Clitellio arenarius* was particularly common in the habitat and at the time of collection (July–August) appeared to form the bulk of the food.

Living prey is detected by the eye-spots and a starved *Lineus* will respond to animals moving within 2 cm. of the head. Dead or injured animals emitting decomposition products or body fluids can be located at greater distances and here it is presumably chemoreceptors in the cephalic ciliated grooves which are stimulated. Detection of living prey is followed by immediate eversion of the proboscis [1] through the proboscis pore at the anterior tip of the body, and this occurs with such explosive force that as the proboscis strikes the prey it becomes coiled around it in a tight spiral grip. It does not penetrate the prey, since it lacks stylets or similar piercing organs, but the tightness of its grip may rupture the integument and cause loss of body fluids or gut contents. The grip is aided by sticky secretions from the proboscis epithelium and immediately it is secured the proboscis begins to retract and draws the prey, usually struggling violently, back towards the mouth. This lies ventrally 2–3 mm. behind the proboscis pore, and as the prey is drawn towards it the body anterior to the mouth is raised and extended until it can grasp the prey by curling downwards over it. This movement of the anterior tip of the body continues downwards and backwards and forces the prey into the mouth which gapes open to receive it. The proboscis then gradually relinquishes its grip and withdraws into the rhynchocoel as movements of the mouth, aided by contractions of the anterior body musculature, force the prey into the gut. Ingestion of small animals is completed in 15 to 20 seconds but with larger prey, such as annelids one-third to one-half the length of the feeding individual, it may take as long as 30 minutes, and in such cases the first part to be swallowed is partially disintegrated before ingestion is complete. Small animals usually die within seconds of entering the gut, but active errant polychaetes with armored jaws may survive long enough either to force their way to the exterior through the gut and body walls or propel themselves down the length of the gut to emerge unharmed at the anus. This is particularly liable to happen when the prey is ingested head first, but in the majority of cases the proboscis seizes an animal about its middle and consequently draws it back to the mouth bent upon itself in the shape of a U. It is then ingested in this form and is unable to escape from the gut before being killed.

During capture and ingestion *Lineus* extends to its fullest length and produces copious sticky mucoid secretions from the ventral surface. This enables a firm hold to be retained upon the substratum whilst dealing with the prey, and even if the latter is partially buried it can be drawn from its retreat and swallowed without causing the *Lineus* to shift position.

Inert masses of food, such as animal remains or the test foods used in this investigation, do not stimulate eversion of the proboscis but are seized directly by the mouth and swallowed piecemeal.

The structure of the gut

The gut consists of three histologically distinct regions, namely the mouth and buccal cavity, the foregut, and the intestine. It runs the length of the body from mouth to anus without coiling, is ciliated throughout and lacks both multicellular glands and musculature.

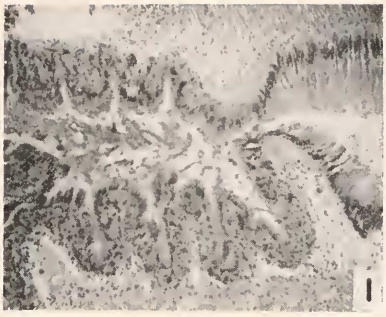
[1] Details of the proboscis and the mechanism of its eversion are given by Hyman (1951) and are not included here.

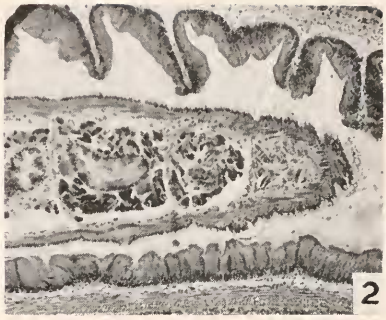
The mouth consists of a ventral subterminal invagination of the epidermis some 200 μ deep and opening directly into the buccal cavity. It is fringed with large cilia and externally has a lobed appearance due to folds in its walls which allow expansion during ingestion. The invaginated epidermis contains acidophil, P.A.S.- and Alcian blue-positive gland cells whose secretions probably facilitate passage of food, and the entire mouth region is surrounded by concentrations of similar sub-epidermal gland cells. The buccal cavity is lined by ciliated cuboidal cells 10–12 μ tall and these are backed by masses of acidophil and basophil gland cells, the majority of which stain with Alcian blue and appear to have the same function as those around the mouth. The walls of the cavity are much folded and ascend diagonally backwards to become continuous with those of the foregut beneath the proboscis sheath.

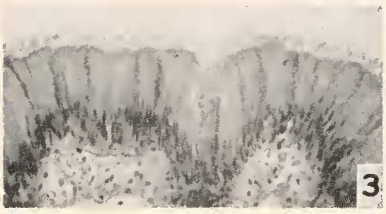
The foregut (Fig. 1) runs posteriorly for one-tenth the length of the animal and its walls are considerably thickened, especially ventrally where the wall may be up to 300 μ in depth. They are thrown into small simple folds and consist of a single layer of ciliated cuboidal cells, 10–12 μ tall, lining the lumen and lying upon acidophil syncytial tissue containing numerous gland cells, free nuclei and occasional large lacunae. In the anterior portion the gland cells consist of P.A.S.- and Alcian blue-positive acidophils and basophils in approximately equal amounts, together with a number of others which are intensely basophilic but give no reaction to Alcian blue. The proportion of the latter increases along the length of the foregut to the median portion where all the gland cells are of this type. The gut wall then gradually decreases in thickness and gland cell content as it nears the intestine and terminates in a constriction (Fig. 1) separating the latter from the foregut.

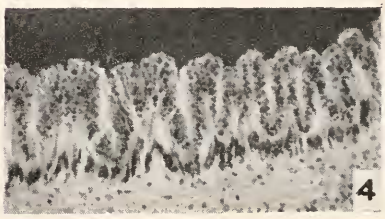
The intestine is the longest part of the gut and runs from its junction with the foregut in the anterior part of the body direct to the anus at the extreme posterior end. It bears paired and serially repeated lateral pouches or caeca throughout its length, apart from a short unpouched region immediately before the anus. The intestinal wall or gastrodermis (Fig. 3) is made up of two types of cells arranged in a single layer upon a thin basement membrane. The larger and more numerous of these are attenuated columnar cells, 50–55 μ tall and 8 μ wide, with granular basophilic cytoplasm containing various acidophil inclusions and basal vesicular nuclei. The free distal borders of the cells bear cilia which in unfed individuals are of uniform appearance and size, but in the presence of digesting food the cilia lose their uniformity and coalesce into pseudopodia-like processes which extend out into the lumen (Fig. 5). This peculiar modification of the cilia is correlated with entry of food material into the cells and is dealt with later.

The second type of cell found in the gastrodermis is glandular and occurs between the bases of the columnar cells. These gland cells (Figs. 2 and 3) are 40–50 μ tall and 5–6 μ wide, unciliated and contain up to thirty acidophil proteinaceous spheres, each 0.5 μ in diameter, which are discharged into the intestinal lumen when food enters from the foregut. They are most numerous in the anterior part of the intestine, where there may be as many as one gland cell to every three columnar, but this ratio is graded down the length of the intestine to approximately one in twenty in the middle region and one in fifty or more beyond until the gland cells disappear, finally, in the short unpouched region before the anus.









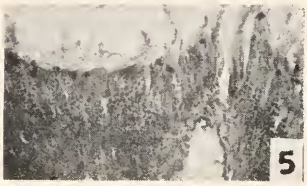


FIGURE 1. Longitudinal section of *Lineus* showing the posterior portion of the foregut (left) and the constriction which separates this from the intestine (right). Haematoxylin and eosin. Scale: 1 cm. = 50 μ.

FIGURE 2. Longitudinal section of the intestine in *Lineus* showing part of a newly ingested *Clitellio* lying intact and undamaged in the lumen. Acidophil gland cells are prominent in the gastrodermis in the lower portion of the figure. Haematoxylin and eosin. Scale: 1 cm. = 100 μ.

FIGURE 3. A portion of the gastrodermis in *Lineus* showing ciliated columnar cells interspersed with darker acidophil gland cells. Intestine empty. Haematoxylin and eosin. Scale: 1 cm. = 50 μ.

FIGURE 4. The gastrodermis in *Lineus* 30 minutes after a meal of frog erythrocytes. The intestinal lumen (top) contains a homogeneous mass of digested haemolyzed erythrocytes which stains heavily with Feulgen and almost obscures the ciliary processes. The columnar cells are loaded with engulfed spherical masses identical in nature with the contents of the lumen. Feulgen and light green. Scale: 1 cm. = 50 μ.

FIGURE 5. The gastrodermis in *Lineus* 30 minutes after a meal of raw starch grains. The cilia have coalesced into pseudopodia-like processes and a few starch grains already engulfed are ranged along the distal margins of the columnar cells. Lugol. Scale: 1 cm. = 50 μ.

FIGURE 6. The gastrodermis in *Lineus* 60 minutes after a meal of raw starch grains. The columnar cells are loaded with grains, many of which are as yet unchanged and still exhibit the characteristic black cross in polarized light. The lumen contains occasional free grains and on the left portions of two gregarine trophozoites with prominent nuclei. Section stained with haematoxylin and eosin and photographed by polarized light. Scale: 1 cm. = 40 μ.

The lateral pouches have the same structure as the rest of the intestine and are merely simple extensions to increase its area, not specialized digestive caeca.

The course of digestion

Ingested food passes rapidly through the buccal cavity into the foregut where it is held for a few seconds before its passage intact into the intestine (Fig. 2). Living food usually dies during the brief pause in the foregut and this is due, no doubt, to acid secretions from the numerous basophilic gland cells present here, for when particles of fish muscle stained with bromo-cresol purple or chlor-phenol red were fed, their pH value fell from 7.0 to 5.5 as they passed through the foregut, and sections of newly fed individuals showed the majority of the glands to be discharged.

There is no trituration or break-up of the food in the foregut but digestion begins immediately it enters the intestine. A series of individuals fixed at intervals after ingestion of the oligochaete *Clitellio* showed that as early as fifteen minutes after feeding the gland cells had discharged their spheres and digestion was well advanced. The *Clitellio* lay in the main median portion of the intestine with the epidermis deeply eroded and the entire body starting to fragment. The cilia of the columnar cells were still uniform in appearance and apparently creating currents in the gut contents to distribute the fragmenting food, for pieces of tissue were already passing into the lateral pouches. Digestion progressed rapidly with time and 30 minutes after feeding the intestine contained a heterogeneous mass of heavily eroded pieces of tissue, intact and fragmented setae, nephridia (which resisted digestion for longer than other tissues and stood out from these with surprising clarity) and diatoms, algal chains, etc. released from the oligochaete gut. The gastrodermal cilia were now beginning to lose their uniformity and coalesce into pseudopodia-like processes stretching into the lumen of the intestine, whilst semidigested material from the latter was appearing as acidophil spheres in the distal portions of the columnar cells bearing these structures. These spheres passed back deeper into the cells and their number increased rapidly with time. Sixty minutes after feeding, the material in the lumen was almost homogeneous, with setal fragments and diatom cases being the only recognizable elements in it, whilst the columnar cells of the gastrodermis were packed with spheres of food undergoing intracellular digestion. The spheres decreased in size and affinity for stains (especially the Millon reagent for protein) as they passed down the cells to disappear finally in the basal region, the cells presumably then passing the products of digestion to other tissues whilst taking up more semidigested material distally until the lumen was emptied. This occurred some six hours after feeding and the amount of intracellular material then rapidly decreased. After a further three hours the columnar cells contained only a few acidophil inclusions, their cilia had resumed their normal shape and size, and the gland cells were again full of enzymatic spheres. Indigestible residues were collected in the short unpouched region of the intestine near the anus, being swept there, probably, by the reconstituted cilia, and observations on living specimens showed that they were expelled eventually by a sudden contraction of the posterior body musculature.

A parallel series of feeding experiments, using fish muscle stained with indicators, showed that the initial drop in pH as the food passes through the foregut

is maintained in the intestine during digestion. In some cases it fell even further, to pH 5.0, and when sufficient indicator-stained material entered the columnar cells to be visible in neutral saline squashes, the intracellular digestion was seen to be progressing in a similarly acid medium of pH 5.0–5.5.

It was not clear from the *Clitellio*-fed series how semidigested material enters the columnar cells, but the pseudopodia-like appearance of the coalesced cilia and spherical compact form of the material when within the cells suggested a form of phagocytosis. This possibility was investigated by feeding *Lineus* on frog erythrocytes and raw, optically active starch grains made palatable by mixing with frog plasma, to ascertain whether such discrete particles were in fact taken into the columnar cells. In the series fed on erythrocytes, however, haemolysis occurred as they entered the intestine, the break-up including the majority of the nuclei, and 30 minutes after feeding the lumen contained a semidigested mass which gave a strong reaction with Feulgen, due to released nuclear materials, and with the benzidine-peroxide reaction for haemoglobin. The cilia had coalesced into the usual processes and many of the cells contained spherical masses with the same staining properties as the material in the lumen (Fig. 4). These apparently phagocytosed masses passed back into the cells as more appeared distally, and gradually decreased in size and their reaction to Feulgen and benzidine-peroxide as intracellular digestion progressed. Digestion of the blood meal was completed in six hours and the intracellular spheres disappeared without leaving residues of haematin or other insoluble pigments from the degradation of the haemoglobin.

Final confirmation of the occurrence of phagocytosis came from the series fed on starch grains. Thirty minutes after feeding the cilia had formed filamentous processes extending into the lumen, and a few intact grains, staining blue with Lugol and still exhibiting the characteristic black cross in polarized light, had already been taken into the cells and were ranged along their distal margins (Fig. 5). The number of such grains increased rapidly and 60 minutes after feeding packed the columnar cells (Fig. 6). Only grains 5 μ or less in diameter were engulfed and larger ones remained in the lumen where they gradually lost their optical activity, fragmented and stained brown with Lugol. The fragments then passed into the cells and joined the previously engulfed grains which were undergoing intracellular digestion, losing their identity and disappearing towards the bases of the cells.

Parasites of the gut

Approximately 75% of the *Lineus* examined contained in the intestine an acephaline eugregarine identified as *Urospora nemertes* (Koelliker). Trophozoites (Fig. 6) 150–180 μ long and 15–20 μ wide, with basophil, P.A.S.-positive cytoplasm and prominent nuclei, occurred in all parts of the lumen, and the intracellular stages, strikingly prominent with P.A.S., were common in the columnar cells. The gregarine did not appear to harm *Lineus* in any way, apart from a few occasions when infected columnar cells reacted against developing intracellular stages and caused them to degenerate into masses of yellowish brown crystals. Such cells then burst, either *in situ* or after being shed into the lumen, and the crystals were eliminated with the faeces.

The food reserves

Fat forms the principal food reserve in *Lineus* and occurs as intracellular globules up to 5 μ in diameter in the parenchyma and, to a lesser extent, in the columnar cells of the intestine. There are no specific protein reserves and the only demonstrable carbohydrate reserve is in the form of tiny granules of glycogen scattered throughout the parenchyma, musculature, and columnar cells.

Discussion

The main points of interest in the nutrition of *Lineus ruber* lie in the feeding mechanism and the digestive processes. In the case of the former a simple but effective method of capturing the food, supplemented by slight modification of the anterior portion of the alimentary canal into a thick-walled glandular foregut for its reception and killing, enables this rhynchocoelan to prey successfully upon animals far more active and elaborate than itself. In this respect *Lineus* resembles the turbellarian flatworms where similarly simple feeding mechanisms make available prey ranging from protozoa to molluscs and tunicates (Jennings, 1957; 1959a). In the Turbellaria it is the pharynx which forms the principal element of the feeding mechanism and this organ is thus analogous in function to the rhynchocoelan proboscis as seen in *Lineus*. The only disadvantage apparent in the type of feeding found in *Lineus* is the possibility of escape by the prey before the secretions of the foregut can take effect but this is overcome, no doubt, in those rhynchocoelans which possess a proboscis armed with stylets and poison glands by killing or paralyzing the prey at the moment of capture.

Digestion in *Lineus* follows a pattern observed in other Acoelomata (Jennings, 1957; 1959b) in that both extracellular and intracellular processes are concerned, but here the intestinal wall is ciliated and consequently the semidigested food would be expected to enter by absorption. In fact, however, it enters by a form of phagocytosis, as is proved beyond doubt by the appearance in the columnar cells of starch grains which retain their form and optical activity after entry and so must have passed into the cells as solid discrete particles. This method of taking material into the columnar cells involves temporary modifications in the form and behavior of the cilia during the digestion of each meal, and the protoplasmic pseudopodia-like processes formed from the coalescence of neighboring cilia are probably concerned in the engulfing of semidigested food, although this has not been observed histologically. The need for the intestine to be ciliated probably arises from its length and the absence of musculature, which together create a need for some method of distributing fragmenting food in the early stages of digestion and collecting residues near the anus at the end. Contractions of the body musculature appear to be insufficient for anything but the final expulsion of the collected residues and hence ciliary currents are used. The reason for the retention of phagocytic uptake of food under these conditions is unknown, for there is no apparent reason why extracellular digestion should not be carried to a point where the semidigested food is soluble enough for absorption, and this presents an interesting subject for further investigations.

Summary

1. The rhynchocoelan *Lineus ruber* feeds on small annelids and crustaceans which are captured by the unarmed proboscis and swallowed whole.

2. The alimentary canal is differentiated into three regions: a buccal cavity, a foregut where the prey is killed by acid secretions, and an intestine where it is digested.

3. Digestion is the result of both extracellular and intracellular processes and occurs in an acid medium of pH 5.0–5.5. The enzymes responsible for the initial extracellular breakdown come from gland cells in the intestinal wall and digestion is completed within the columnar cells of the latter. Semidigested food enters these columnar cells by a phagocytic process and this involves temporary modifications in the form and function of their cilia.

4. The food reserves consist of fat deposits in the parenchyma and, to a lesser extent, in the columnar cells of the intestine.

LITERATURE CITED

Coe, W. R., 1943. Biology of the nemerteans of the Atlantic coast of North America. *Trans. Conn. Acad. Arts Sci.*, **35**: 129–328.

Hyman, L. H., 1951. The Invertebrates. Vol. II: Platyhelminthes and Rhynchocoela. McGraw-Hill Book Co., Inc., New York.

Jennings, J. B., 1957. Studies on feeding, digestion and food storage in free-living flatworms (Platyhelminthes: Turbellaria). *Biol. Bull.*, **112**: 63–80.

Jennings, J. B., 1959a. Observations on the nutrition of the land planarian *Orthodemus terrestris* (O. F. Müller). *Biol. Bull.*, **117**: 119–124.

Jennings, J. B., 1959b. Studies on digestion in the monogenetic trematode *Polystoma integerrimum*. *J. Helminth.*, **33**: 197–204.

Reisinger, E., 1926. Nemertini. *In:* P. Schulze (ed.), *Biologie der Tiere Deutschlands*, Lief. **17**.

Wilson, C. B., 1900. The habits and early development of *Cerebratulus lacteus* (Verrill). *Quart. J. Micr. Sci.*, **43**: 97–198.